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Pineal-Induced Depression of Free Thyroxine in Syrian Hamsters

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Blind hamsters had no alteration of the dialyzable fraction of serum thyroxine (T4) but had depressed total and free T4 concentrations compared to controls. Prevention of the effects of blinding by pinealectomy indicates pineal influence on circulating free T4 concentration. Parallel changes in free T4 and the free T4 index indicate adequacy of the index in representing pineal-induced changes in free T4.

Key words: pineal, free thyroxine, thyroxine, hamsters, blinding

INTRODUCTION

When Syrian hamsters are exposed to a short photoperiod (less than 12 hr light per day) for 2–3 months or are subjected to bilateral orbital enucleation, their circulating total thyroxine (T4) concentration falls. This effect is brought about by the pineal gland; pinealectomy prevents it [Vriend et al., 1979; Vriend, 1983]. In the above and other studies [G.M. Vaughan et al., 1982, M.K. Vaughan et al., 1982] of light restriction, the pineal, and T4 in this species the status of circulating free T4 has been approached by

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simultaneous observations of the free T4 index (FT4I), which depends on the in vitro uptake (T3U) of added radiotracer triiodothyronine (T3) from the serum onto a solid matrix. The FT4I (T4 × T3U) thus contained a correction for possible alterations of protein binding of T4 and provided an indirect assessment of its free concentration. Because the FT4I changed with the same pattern exhibited by T4 in the above studies, it was tentatively concluded that the pineal gland, in the condition of light deprivation, is capable of influencing circulating free T4 concentration (FT4).

However, the general use of the FT4I to represent changes of FT4 has recently been called into question. For example, in a number of conditions and species, including various nonthyroidal illnesses in humans [Chopra et al., 1979, 1984; Woeber and Maddux, 1981; Oppenheimer et al., 1982] and burn injury in rats [Shirani et al., 1985], human [Vaughan et al., 1985a], and hamsters [Vaughan et al., 1985b], the total T4 and FT4I were often depressed but the free or dialyzable fraction of T4 (T4DF) was elevated out of proportion to any change in T3U. This often resulted in free T4 concentrations (FT4 = T4 × T4DF) that were not depressed or were less depressed than predicted by the FT4I in these conditions. The T4DF is that portion of radiotracer T4 that crosses a protein-impermeable membrane from the serum into buffer without involvement of a solid matrix. Thus such discrepancy between the T3U and the T4DF (and hence between the FT4I and FT4) might be due to a circulating factor in some conditions that not only inhibits binding of T4 to serum proteins but also to the solid matrix of the T3U test [Oppenheimer et al., 1982]. It is thus necessary to show that, in the experimental condition under study, the changes in FT4I do reflect those of FT4 directly assessed by observations of the T4DF.

Because of these considerations, in the present study we have assessed whether or not the pineal gland, in the condition of its activation by blindness in Syrian hamsters, can influence free T4 concentration.

MATERIALS AND METHODS

Male golden hamsters, *Mesocricetus auratus*, were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts), at about 90 gm body weight (young adults), maintained in our animal quarters in a light/dark (L/D) cycle of 14/10 hr at 21°C with lights on at 0700 hr and given standard laboratory chow and tap water ad libitum. After 2 weeks, they were divided into three groups, according to surgical procedures performed under pentobarbital anesthesia: sham-pinealectomy (SHPX), blinding by bilateral orbital enucleation together with sham-pinealectomy (BL+SHPX), and blinding plus pinealectomy (BL+PX). Pinealectomy was performed according to the method of Hoffman and Reiter [1965], and the sham procedure involved replacing the disc of skull bone without performing pinealectomy. The animals were maintained in the L/D 14/10 cycle until sacrifice by guillotine 11 weeks after surgery, when trunk serum was obtained and both testes and the dorsal prostate were cleaned of fat and connective tissue and weighed. Successful pinealectomy without disruption of the brain

structures in the vicinity was verified grossly at autopsy. T4 was measured by radioimmunoassay and T3U by radioassay (charcoal matrix) with kits from Diagnostic Products (Los Angeles, California). The dialyzable fraction of T4 (T4DF) was determined in duplicate serum aliquots at the Nichols Institute, San Juan Capistrano, California, by equilibrium dialysis at 37°C [Sterling and Brenner, 1966]. The FT4I was calculated as the product of T4 and T3U, and the FT4 as the product of T4 and T4DF (Sterling and Brenner, 1966). Data were analysed by the Student-Newman-Keuls test [Bruning and Kintz, 1977] and analysis of covariance [Dixon, 1983].

RESULTS

Reduction of testicular and prostatic mass was observed in the blind animals, an effect obliterated by additional pinealectomy (Fig. 1). Blinding produced no detectable effect on T3U or T4DF, though a small reduction of mean T3U was seen in the BL+PX group (Table 1). T4, FT4I, and FT4 were all depressed after blinding, unless the animals were additionally pinealectomized (Fig. 1). The correlation of FT4I with FT4 was significant (Fig. 1), and there were no significant differences among slopes for individual groups (not shown).

DISCUSSION

Compared to values in controls, lower mean serum T4 without major alteration of T3U has been reported in blind humans [Dieckhues, 1974], an effect mediated by the pineal gland in Syrian hamsters [Vriend et al., 1977; Vriend et al., 1979; G.M. Vaughan, et al. 1982; M.K. Vaughan, et al., 1982; Vriend, 1983]. We now report that free T4 concentration is depressed by blinding and that this effect appears to be mediated by the pineal gland. The small reduction of mean T3U in BL+PX is not explained, but blinding alone appeared to have no effect on T3U, and FT4I appeared to be a relatively reliable index of FT4 (Fig. 1).

The observed blinding-induced suppression of the reproductive system and the prevention of this by pinealectomy serve to indicate that the animals in this study exhibited the expected pineal-mediated reproductive responses [Reiter, 1980] and thus adequately represent the usual hamster model for demonstrating pineal function. The parallel changes between the reproductive system and circulating T4 have been noted previously, and the possibility has been considered that the changes in T4 passively result from pineal-induced hypogonadism and the possible resultant effects on T4 serum binding proteins [Vriend et al., 1977, 1979; G.M. Vaughan et al., 1982]. It was interpreted that the effects of the pineal on T4 and free T4 were likely independent of such a potential effect of hypogonadism, because the FT4I varied with the same pattern as did total T4. This interpretation is confirmed in the present study, which shows that the free fraction (T4DF) of T4 and hence its serum transport binding in the hamster was not significantly altered in the presence of pineal-induced hypogonadism and that, like T4 and FT4I,

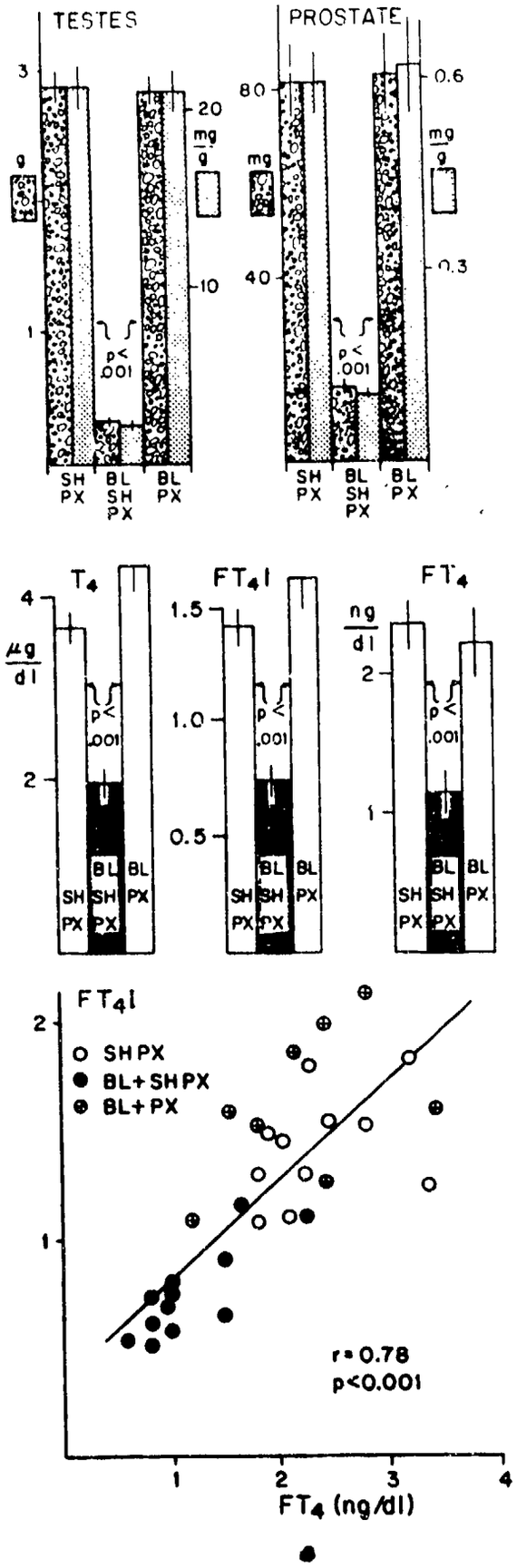

Fig. 1. Testicular and prostate weights as absolute (g) or relative to body weight (mg/g), T4, FT4I, and FT4 after sham pinealectomy (SHPX), blinding (BL + SHPX), or combined blinding and pinealectomy (BL + PX). Means ± SE (top and middle panels) and rectilinear regression (bottom panel).

TABLE 1. T3U and T4DF After Sham Pinealectomy (SHPX), Blinding (BL), and/or Pinealectomy (PX)

	SHPX	BL+SHPX	BL+PX
n in group	11	12	8
T3U (%)	38.04	38.66	36.63**
	±.319	±.293	±.404
T4DF (%)	.0638	.0581	.0504
	±.004	±.004	±.006

**P < 0.01 vs. SHPX and BL+SHPX.
Means ± SE.

FT4 was actually depressed in this condition. Furthermore, it was previously shown that the depressed T4 in blind hamsters was not a result of their gonadal regression, because castration alone did not alter serum T4 [Vriend et al., 1979]. Of course, the possibility remains that the pineal-dependency of the blinding-induced suppressions of both the reproductive and thyroidal systems results from some pineal-dependent common central neural control mechanism [Vriend, 1983].

The magnitude of the dialyzable fraction (T4DF) in hamsters appears to be similar to that in humans [Sterling and Brenner, 1966]. Because humans with altered levels of serum thyroxine-binding protein (and consequently abnormal total serum T4 concentration) often have normal free (dialyzable) concentrations but are euthyroid, it is thought that the free concentration is the bioavailable portion important for hormone action [Ingbar and Woeber, 1981]. Although somewhat more than the dialyzable fraction can be transported into some rat tissues [Pardridge, 1981], the precise fraction of T4 that is active in hamsters particularly at the level of the pituitary has not been elucidated. In the rat and human with primary reduction of thyroid function, it appears that T4 rather than T3 in the circulation determines feedback in the pituitary for thyrotrophin (TSH) secretion, via local T4 to T3 conversion [Connors and Hedge, 1981; Larsen, 1982]. The observation that both total and free T4 in serum are lower in blind than in control hamsters is important, because it indicates that the pineal gland can induce a condition in which bioavailable T4 in the circulation is reduced, a finding previously only suspected on the basis of indirect FT4I results [Vriend et al., 1977]. Whether or not the reduced FT4 results in other physiologic changes in these animals remains to be determined.

The site of the suppressive action of the pineal on serum T4 might be some element of the hypothalamopituitary unit (central), the thyroid, the organs responsible for T4 conversion or degradation (peripheral), or a combination of the above. TSH was not determined in the present animals because of an insufficient quantity of serum. As reviewed previously for the Syrian hamster [G.M. Vaughan, et al., 1982], in animals blind for 2–3 months, mean TSH is not consistently altered at a time when serum T4 is always low. Thus it is not unreasonable to suggest that the effect of the pineal is at least partly at the level of the pituitary or hypothalamus [Vriend, 1983], causing an inadequacy in the compensatory elevation of TSH, which normally should be a response to a fall in free T4 concentration [Larsen, 1982].

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